NTP
National Toxicology Program

Workshop Report: Role of Environmental Chemicals in the Development of Diabetes and Obesity (January 11-13, 2011)

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NTP Board of Scientific Counselors Meeting
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National Institute of Environmental Health Sciences / National Institutes of Health
National Institute for Occupational Safety and Health / Centers for Disease Control
National Center for Toxicological Research / Food and Drug Administration

Diabetes and Obesity Are Major Risks to Public Health

- 12.9% of people ≥20 of age in US estimated to have diabetes
- Worldwide 220 million, expected 366 million by 2030 (WHO)
- ~70% of type 2 diabetes risk attributed to overweight/obesity
  - ~30% not accounted for by body weight
- 33.8% of US adults are obese
- Prevalence of obesity almost tripled since 1980 in 2-19 year olds
- Increased obesity in preschool children aged 2-5 years from 5% in 1976-1980 to 10.4% in 2007-2008

Overall Goals of Workshop

- Evaluate the science associating exposure to certain chemicals or chemical classes with development of diabetes or obesity in humans

Arsenic	Persistent organic pollutants (POPs)
Bisphenol A (BPA)	Pesticides
Trialkyltins ("Organotins")	Phthalates
Maternal Smoking	Nicotine

- Provide input to NTP and NIEHS for development of a research agenda

Format

- Bring together diverse expertise
  - Epidemiologist, toxicologists, bioinformaticists, and experts in the pathobiology of disease
- Mostly breakout group deliberations
  - Focus on diabetes and/or obesity, depending on chemical
  - Charge questions and background materials tailored to suit each breakout group
  - General charge questions
    - Evaluate evaluate the strength/weaknesses, consistency, and biological plausibility
    - Identify the most useful and relevant endpoints and best practices
    - Identify data gaps and research needs
- Workshop materials at http://ntp.niehs.nih.gov/go/36433

Consider Data from Tox21 High Throughput Screening

- Collaborative program among EPA, FDA, NIEHS/NTP, and NIH Chemical Genomics Center
  - Includes a variety of assay platforms/technologies
- Tox21 data incorporated into several chapters and sessions
  - Introduce researchers to Tox21
    - Mostly data from Phase 1 of EPA's ToxCast™ program
  - Stimulate discussion on how to best assess applications of Tox21 data
    - Help determine biological plausibility of reported effects
    - Use to identify research questions
  - Identify additional assay targets/technologies for diabetes/obesity

Major Conclusions

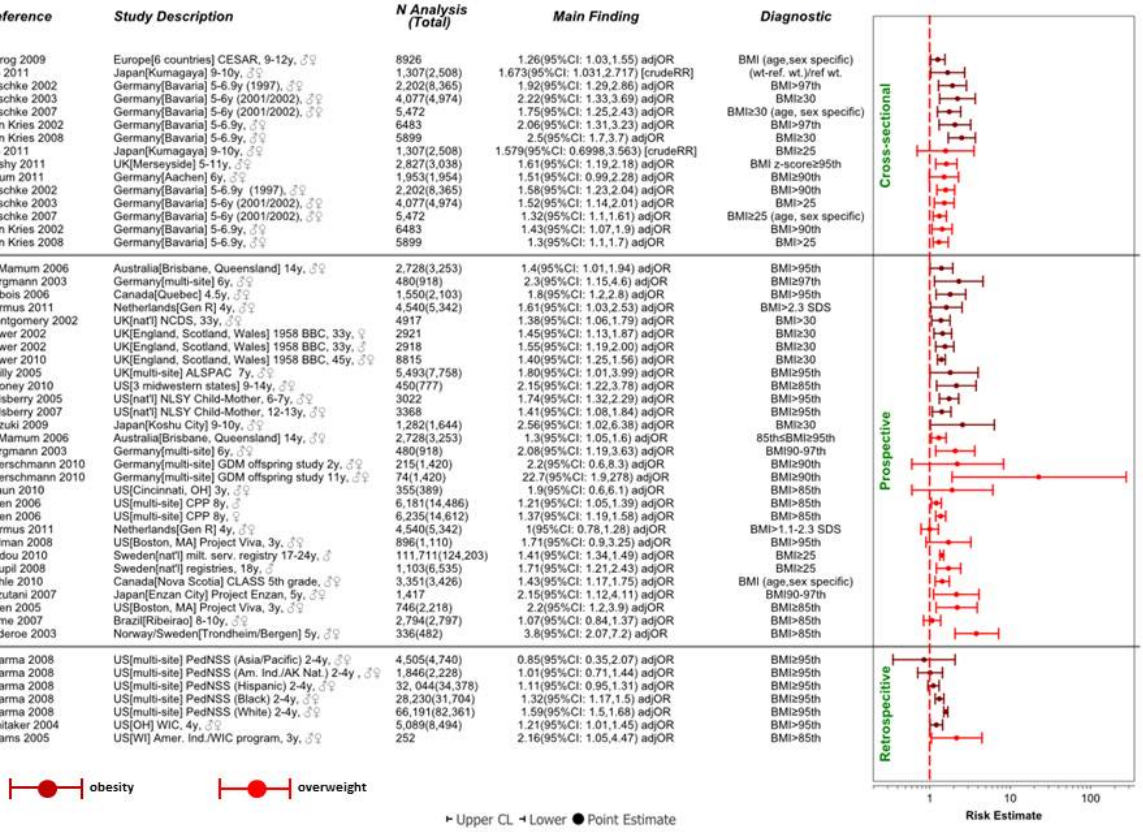
- Positive associations in epidemiological studies
  - Maternal smoking during pregnancy and childhood obesity
  - Arsenic in areas of high exposure and diabetes
  - Certain chlorinated persistent organic pollutants and diabetes (PCBs, DDT/DDE, Agent Orange/TCDD in Vietnam vets, trans-nonachlor)
- Support for biological plausibility from animal and mechanistic studies
  - Organotins as developmental "obesogens"
  - BPA and altered glucose homeostasis and adiposity-related effects
  - Organophosphate and other pesticides
- Tox21 an intriguing tool for assessing biological plausibility and developing research questions

Maternal Smoking and Childhood Obesity

- Maternal smoking during pregnancy is associated with increased risk of childhood overweight/obesity
  - Animal studies with nicotine reproduce "to a large extent" metabolic changes seen in the children of mothers who smoke
  - Provides support for plausibility of "obesogen" hypothesis
  - Mechanistic studies suggest biologically plausible associations of nicotine with the disruption of pathways important in obesity and diabetes (e.g., effects on beta cell mass and function)
  - Many "disease pathways" remain unexplored
    - Insulin signaling, feeding behavior, peripheral inflammation, insulin resistance, etc.
  - Less support for linkage to Type 1 diabetes in human studies

Maternal Smoking During Pregnancy and Childhood Overweight and Obesity

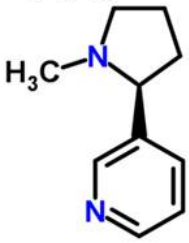
Reference	Study Description	N Analysis (Total)	Main Finding	Diagnostic
Gorog 2009	Europe[6 countries] CESAR, 9-12y, ♂♀	8926	1.26(95%CI: 1.03,1.55) adjOR	BMI (age,sex specific)
Ino 2011	Japan[Kumagaya] 9-10y, ♂♀	1,307(2,508)	1.673(95%CI: 1.031,2.717) [crudeRR]	(wt-ref. wt.)/ref wt.
Toschke 2002	Germany[Bavaria] 5-6.9y (1997), ♂♀	2,202(8,365)	1.92(95%CI: 1.29,2.86) adjOR	BMI>97th
Toschke 2003	Germany[Bavaria] 5-6y (2001/2002), ♂♀	4,077(4,974)	2.22(95%CI: 1.33,3.69) adjOR	BMI≥30
Toschke 2007	Germany[Bavaria] 5-6y (2001/2002), ♂♀	5,472	1.75(95%CI: 1.25,2.43) adjOR	BMI≥30 (age, sex specific)
Von Kries 2002	Germany[Bavaria] 5-6.9y, ♂♀	6483	2.06(95%CI: 1.31,3.23) adjOR	BMI>97th
Von Kries 2008	Germany[Bavaria] 5-6.9y, ♂♀	5899	2.5(95%CI: 1.7,3.7) adjOR	BMI≥30
Ino 2011	Japan[Kumagaya] 9-10y, ♂♀	1,307(2,508)	1.579(95%CI: 0.6998,3.563) [crudeRR]	BMI≥25
Koshy 2011	UK[Merseyside] 5-11y, ♂♀	2,827(3,038)	1.61(95%CI: 1.19,2.18) adjOR	BMI z-score≥95th
Raum 2011	Germany[Aachen] 6y, ♂♀	1,953(1,954)	1.51(95%CI: 0.99,2.28) adjOR	BMI≥90th
Toschke 2002	Germany[Bavaria] 5-6.9y (1997), ♂♀	2,202(8,365)	1.58(95%CI: 1.23,2.04) adjOR	BMI>90th
Toschke 2003	Germany[Bavaria] 5-6y (2001/2002), ♂♀	4,077(4,974)	1.52(95%CI: 1.14,2.01) adjOR	BMI>25
Toschke 2007	Germany[Bavaria] 5-6y (2001/2002), ♂♀	5,472	1.32(95%CI: 1.1,1.61) adjOR	BMI≥25 (age, sex specific)
Von Kries 2002	Germany[Bavaria] 5-6.9y, ♂♀	6483	1.43(95%CI: 1.07,1.9) adjOR	BMI>90th
Von Kries 2008	Germany[Bavaria] 5-6.9y, ♂♀	5899	1.3(95%CI: 1.1,1.7) adjOR	BMI>25
Al Mamum 2006	Australia[Brisbane, Queensland] 14y, ♂♀	2,728(3,253)	1.4(95%CI: 1.01,1.94) adjOR	BMI>95th
Bergmann 2003	Germany[multi-site] 6y, ♂♀	480(918)	2.3(95%CI: 1.15,4.6) adjOR	BMI≥97th
Dubois 2006	Canada[Quebec] 4.5y, ♂♀	1,550(2,103)	1.8(95%CI: 1.2,2.8) adjOR	BMI>95th
Durmus 2011	Netherlands[Gen R] 4y, ♂♀	4,540(5,342)	1.61(95%CI: 1.03,2.53) adjOR	BMI>2.3 SDS
Montgomery 2002	UK[nat'l] NCDS, 33y, ♂♀	4917	1.38(95%CI: 1.06,1.79) adjOR	BMI>30
Power 2002	UK[England, Scotland, Wales] 1958 BBC, 33y, ♀	2921	1.45(95%CI: 1.13,1.87) adjOR	BMI≥30
Power 2002	UK[England, Scotland, Wales] 1958 BBC, 33y, ♂	2918	1.55(95%CI: 1.19,2.00) adjOR	BMI≥30
Power 2010	UK[England, Scotland, Wales] 1958 BBC, 45y, ♂♀	8815	1.40(95%CI: 1.25,1.56) adjOR	BMI≥30
Reilly 2005	UK[multi-site] ALSPAC 7y, ♂♀	5,493(7,758)	1.80(95%CI: 1.01,3.99) adjOR	BMI≥95th
Rooney 2010	US[3 midwestern states] 9-14y, ♂♀	450(777)	2.15(95%CI: 1.22,3.78) adjOR	BMI≥85th
Salsberry 2005	US[nat'l] NLSY Child-Mother, 6-7y, ♂♀	3022	1.74(95%CI: 1.32,2.29) adjOR	BMI>95th
Salsberry 2007	US[nat'l] NLSY Child-Mother, 12-13y, ♂♀	3368	1.41(95%CI: 1.08,1.84) adjOR	BMI≥95th
Suzuki 2009	Japan[Koshu City] 9-10y, ♂♀	1,282(1,644)	2.56(95%CI: 1.02,6.38) adjOR	BMI≥30
Al Mamum 2006	Australia[Brisbane, Queensland] 14y, ♂♀	2,728(3,253)	1.3(95%CI: 1.05,1.6) adjOR	85thsBMI≥95th
Bergmann 2003	Germany[multi-site] 6y, ♂♀	480(918)	2.08(95%CI: 1.19,3.63) adjOR	BMI90-97th
Boerschmann 2010	Germany[multi-site] GDM offspring study 2y, ♂♀	215(1,420)	2.2(95%CI: 0.6,8.3) adjOR	BMI≥90th
Boerschmann 2010	Germany[multi-site] GDM offspring study 11y, ♂♀	74(1,420)	22.7(95%CI: 1.9,278) adjOR	BMI≥90th
Braun 2010	US[Cincinnati, OH] 3y, ♂♀	355(389)	1.9(95%CI: 0.6,6.1) adjOR	BMI>85th
Chen 2006	US[multi-site] CPP 8y, ♂	6,181(14,486)	1.21(95%CI: 1.05,1.39) adjOR	BMI>85th
Chen 2006	US[multi-site] CPP 8y, ♀	6,235(14,612)	1.37(95%CI: 1.19,1.58) adjOR	BMI>85th
Durmus 2011	Netherlands[Gen R] 4y, ♂♀	4,540(5,342)	1(95%CI: 0.78,1.28) adjOR	BMI>1.1-2.3 SDS
Gillman 2008	US[Boston, MA] Project Viva, 3y, ♂♀	896(1,110)	1.71(95%CI: 0.9,3.25) adjOR	BMI>95th
Iliadou 2010	Sweden[nat'l] milt. serv. registry 17-24y, ♂	111,711(124,203)	1.41(95%CI: 1.34,1.49) adjOR	BMI≥25
Koupil 2008	Sweden[nat'l] registries, 18y, ♂	1,103(6,535)	1.71(95%CI: 1.21,2.43) adjOR	BMI≥25
Kuhle 2010	Canada[Nova Scotia] CLASS 5th grade, ♂♀	3,351(3,426)	1.43(95%CI: 1.17,1.75) adjOR	BMI (age,sex specific)
Mizutani 2007	Japan[Enzan City] Project Enzan, 5y, ♂♀	1,417	2.15(95%CI: 1.12,4.11) adjOR	BMI90-97th
Oken 2005	US[Boston, MA] Project Viva, 3y, ♂♀	746(2,218)	2.2(95%CI: 1.2,3.9) adjOR	BMI≥85th
Tome 2007	Brazil[Ribeirao] 8-10y, ♂♀	2,794(2,797)	1.07(95%CI: 0.84,1.37) adjOR	BMI>85th
Wideroe 2003	Norway/Sweden[Trondheim/Bergen] 5y, ♂♀	336(482)	3.8(95%CI: 2.07,7.2) adjOR	BMI>85th
Sharma 2008	US[multi-site] PedNSS (Asia/Pacific) 2-4y, ♂♀	4,505(4,740)	0.85(95%CI: 0.35,2.07) adjOR	BMI≥95th
Sharma 2008	US[multi-site] PedNSS (Am. Ind./AK Nat.) 2-4y , ♂♀	1,846(2,228)	1.01(95%CI: 0.71,1.44) adjOR	BMI≥95th
Sharma 2008	US[multi-site] PedNSS (Hispanic) 2-4y, ♂♀	32, 044(34,378)	1.11(95%CI: 0.95,1.31) adjOR	BMI≥95th
Sharma 2008	US[multi-site] PedNSS (Black) 2-4y, ♂♀	28,230(31,704)	1.32(95%CI: 1.17,1.5) adjOR	BMI≥95th
Sharma 2008	US[multi-site] PedNSS (White) 2-4y, ♂♀	66,191(82,361)	1.59(95%CI: 1.5,1.68) adjOR	BMI≥95th
Whitaker 2004	US[OH] WIC, 4y, ♂♀	5,089(8,494)	1.21(95%CI: 1.01,1.45) adjOR	BMI>95th
Adams 2005	US[WI] Amer. Ind./WIC program, 3y, ♂♀	252	2.16(95%CI: 1.05,4.47) adjOR	BMI>85th



Nicotine and ToxCast™

- Nicotine not in ToxCast™
- Nicotinic acetylcholine receptor (nAChRs) assay
- Some ToxCast™ Phase 1 compounds interact with key receptor target

Nicotine
54-11-5
$C_{10}H_{14}N_2$ (MW 162.23)

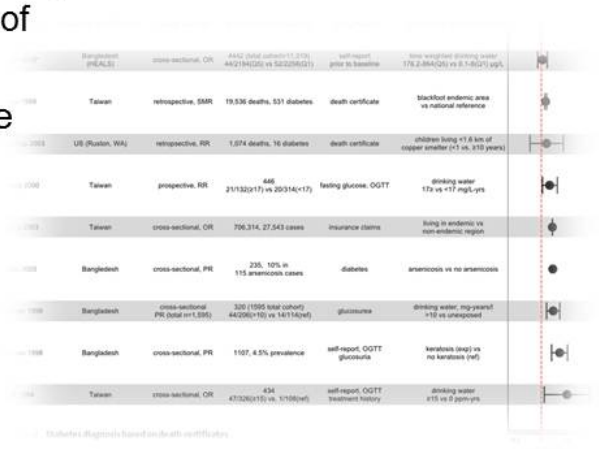


Name	CASRN	NVS_LGIC_hNNR_NBungSens (uM)
Acetamiprid	135410-20-7	5.7
Clothianidin	210880-92-5	30.0
Cyazofamid	120116-88-3	26.0
Imidacloprid	138261-41-3	9.7
Mepiquat chloride	24307-26-4	35.0
Thiacloprid	111988-49-9	4.9

Use: Alkaloid found in the nightshade family of plants (Solanaceae) that constitutes approximately 0.6–3.0% of dry weight of tobacco. Considered the main factor responsible for dependence forming properties for tobacco use
Mechanism: binds to nicotinic acetylcholine receptors (nAChRs)

Arsenic and Diabetes

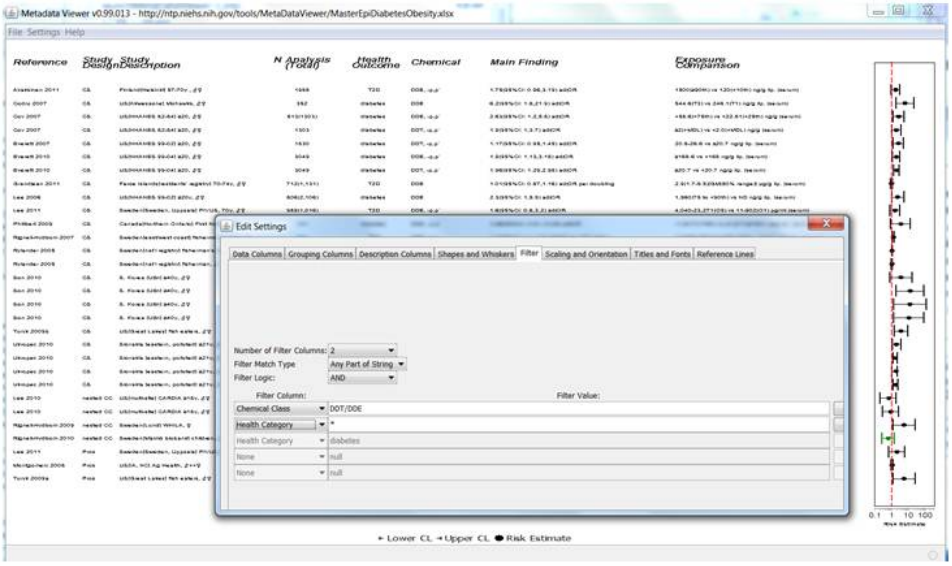
- "Limited" to "sufficient" in support of an association between arsenic and diabetes in populations with high exposure levels
  - Bangladesh and Taiwan
- "Insufficient" evidence for an association with diabetes and arsenic in lower exposure areas, e.g., US, Mexico
  - More recent studies suggestive of an association
- Overall, animal data inconclusive but recent studies support a linkage
  - Mechanistic studies also supportive
- Not tested in Tox21



POPs

- Complex literature, many human studies
- Needed expert input prior to developing text-based chapter
  - ~50 page table of study summaries, ~500 main results
  - Too large of a literature to look for patterns in text-based format
- Developed Meta Data Viewer graphing program to screen studies
  - Developed by Shawn Harris, SRA International

Meta Data Viewer Software



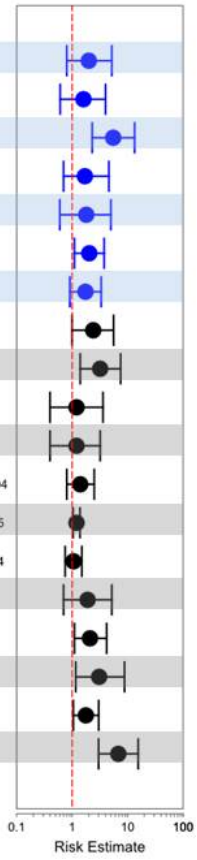
- Web-start at http://ntp.niehs.nih.gov/go/tools_metadataviewer

POPs and Diabetes

- Using the forest plot generator, identified classes of POPs that should be considered together
- Evidence is "sufficient" for an association with diabetes based on collected analyses of cross-sectional, prospective/retrospective, and occupational exposure studies
  - Initial data-mining indicates strongest correlations of diabetes with trans-nonachlor, DDE, and dioxins/dioxin-like chemicals including PCBs
- Animal studies not primary focus of breakout group discussion
  - Existing literature generally considered of limited utility
- Not tested in Tox21

PCBs and Diabetes

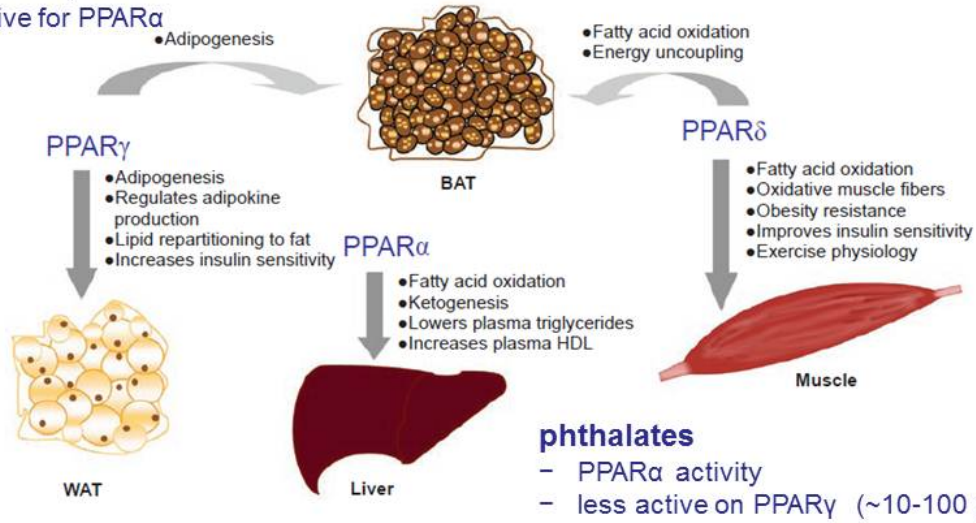
Reference	Study Design	Country and Cohort	N in Analysis (N in Cohort)	Chemical	Exposure Comparison
Lee, 2010	nested case control, OR	US, CARDIA	95 (180)	PCB153	Q2 (205-349) vs. Q1 (≤204) pg/g
Rignell-Hydbom, 2009	nested case control, OR	Sweden, women in WHILA	39 pairs (371)	PCB153	>1790 ppt >7 years vs ≤1790 at baseline
Wang, 2008	nested case control, OR	Taiwan, Yucheng cohort, women	244 (441)	PCBs	121.4 vs. 72.6 ppb, based on chloracne
Wang, 2008	nested case control, OR	Taiwan, Yucheng cohort, men	167 (307)	PCBs	99.4 vs. 53.9 ppb, based on chloracne
Turyk, 2009a	prospective, IDR	US, Great Lakes fish eaters	314 (471)	PCBs	4.3-29.8 vs. <1.6 ng/g wet weight, p-trend=0.37
Vasiliu, 2006	prospective, IDR	US, MI PBB cohort, women	459 (696)	PCBs	5.1-7.0 vs ≤5.0 ppb
Vasiliu, 2006	prospective, IDR	US, MI PBB cohort, men	360 (688)	PCBs	>10 vs ≤5.0 ppb
Codru, 2007	cross-sectional, OR	US, Mohawks near Akwesasne	235 (352)	PCB153	104.4 vs. 59.8 ppb
Codru, 2007	cross-sectional, OR	US, Mohawks near Akwesasne	235 (352)	PCBs	756.2 vs 448.6 ppb
Jorgensen, 2008	cross-sectional, OR	Greenland, ≥ Inuit parent	692	PCBs, dioxin-like	Q4 vs Q1, p-trend=0.37
Jorgensen, 2008	cross-sectional, OR	Greenland, ≥ Inuit parent	692	PCBs, non-dioxin like	Q4 vs Q1, p-trend=0.42
Rignell-Hydbom, 2007	cross-sectional, OR	Sweden, fisherman's wives	543	PCB153	per 100 ng/g ↑ (100ng/g lipid, cases), p-trend=0.004
Rylander, 2005	cross-sectional, OR	Sweden, fishermen	196 (380)	PCB153	per 100 ng/g ↑ (560g/g lipid, cases), p-trend=0.005
Rylander, 2005	cross-sectional, OR	Sweden, fishermen's wives	184 (380)	PCB153	per 100 ng/g ↑ (230ng/g lipid, cases), p-trend=0.94
Turyk, 2009b	cross-sectional, OR	US, Great Lakes fish eaters	503	PCBs	3.6-24.4 vs <0.8 ng/g (p-trend = 0.36)
Turyk, 2009b	cross-sectional, OR	US, Great Lakes fish eaters	503	PCBs, dioxin-like	0.3-1.6 vs <LOD ng/g (p-trend = 0.03)
Uemura, 2008	cross-sectional, OR	Japan, general pop	1003(1374)	PCBs, dioxin-like	≥7.60 to <13 vs. ≤7.60 pg TEQ/g lipid
Ukropec, 2010	cross-sectional, OR	Slovakia, general pop	818 (2047)	PCBs	Q4 (1341-2330) vs. Q1 (148-627) ng/g
Lee, 2006	cross-sectional, OR	US, NHANES 99-02	577 (2,106)	PCB153	164 ppb vs. ND



Peroxisome Proliferator-Activated Receptors (PPARs) – Organotins and Phthalates

organotins

- potent PPARγ and RXRα agonist (~10-100 nM)
- not active for PPARα



phthalates

- PPARα activity
- less active on PPARγ (~10-100 μM)

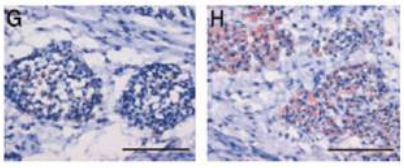
From Wang YX. 2010. PPARs: diverse regulators in energy metabolism and metabolic diseases. Cell Res 20(2):124-137.

Organotins/Phthalates

- Human studies are “insufficient” (phthalates) or nonexistent (organotins) for evaluating an association with diabetes or obesity
- Recent mechanistic studies show potent effects of tributyltin on adipogenesis of adipose-derived stem cells and PPARγ and retinoid X receptor (RXR) activation
  - Mechanistic support stronger for organotins
- Animal phthalate data are problematic because of PPARα contribution; relatively few animal studies on organotins
- ToxCast™ generally consistent with published literature (PPAR, RXR)
- Basis for recommending combination studies from co-occurrence in plastics

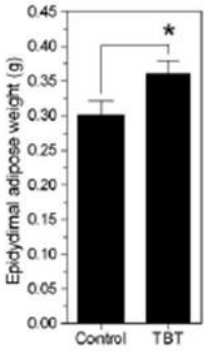
Tributyltin as Inducer of Adipogenesis (Grun et al., 2006)

- C57BL/6 dams treated with 0.5 mg/kg TBT by ip injection on GD12-18; looked at effects in F1 offspring:
  - ↑ lipid accumulation in adipose depots, liver and testis in neonates
  - ↑ epididymal adipose mass in adult male offspring



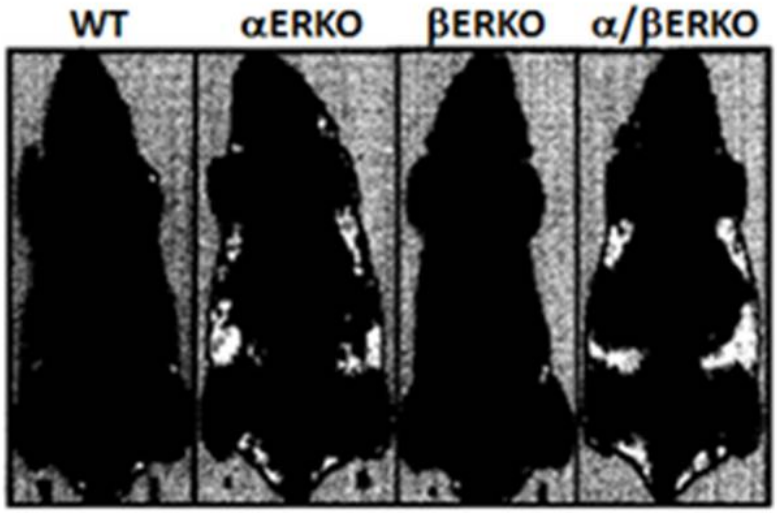
Control TBT

Mammary adipose tissue in neonates
(Oil red O stain)



F1 males at 10 weeks

Body Weight Crude Indicator of Adiposity in Rodents



DXA/image analysis of fat content in 4-month old males; areas with more than 50% fat are shown in white (Ohlsson et al. 2000)

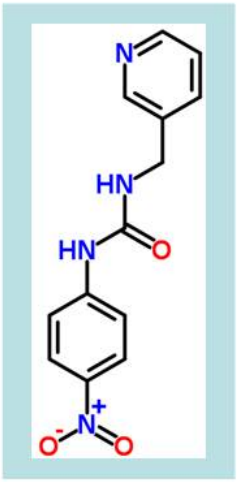
*No difference in body weight (wild-type = 33.0 ± 1.1g; αERKO = 31.6 ± 0.9g)

Pesticides

- Most exploratory aspect of workshop
  - Identify pesticides that can serve as "sign post" for metabolic effects based on human and animal data
    - Published literature and EPA's ToxRefDB
  - Link to ToxCast™ data when possible

Vacor

- Rodenticide used from 1975-1979
  - Banned due to high number of h[illegible]oisonings
  - Can cause Type 1 diabete[illegible]ns
- Damages pancreatic islet c[illegible]imal models
- Nicotinamide antagoni[illegible]
- ROS and inhibitio[illegible]plex 1 of mitochondrial respiration im[illegible]



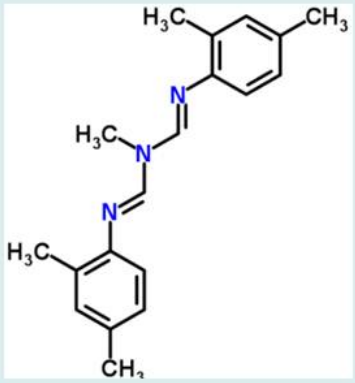
Amitraz

- Insecticide that causes hyperglycemia following poisoning incidents
- Also causes hyperglycemia and reduced insulin secretion in animal models (dogs, rats, mice, honey bees)
- α2-adrenoreceptor agonist
  - α2-adrenoreceptor antagonists block effects

Amitraz

33089-61-1

$C_{19}H_{23}N_3$ (MW 293.41)



Use: amidine insecticide

Mechanism: α2-adrenoreceptors agonist

NTP
National Toxicology Program

Amitraz in ToxCast™

Table 8. Pattern of screening data for Amitraz and other chemicals tested in Phase 1 of ToxCast™ that interacted with same assay targets[1]

CASRN	Name	adrenergic receptor, α-2A (ADRA2A)	adrenergic receptor, α-2A (Adra2a)	monoamine oxidase A (NVS_ENZ_rabI2C)	serotonin receptor 7 (HTR7)	adrenergic receptor, α-2b (Adra2b)	serotonin receptor 1A (Htr1a)
33089-61-1	Amitraz	0.05	0.06	0.16	0.45	1.03	1.8
43222-48-6	Difenzoquat metilsulfate	1.07	3.18	27.1	47.1	0.59	
155569-91-8	Emamectin benzoate	21.3	20.8		4	23.5	
68157-60-8	Forchlorfenuron	22.1	40		48.5		
67747-09-5	Prochloraz		1.83		39.4	4.7	
118134-30-8	Spiroxamine	6.82	29.7		14.4		
119446-68-3	Difenoconazole		2.36			29.5	
76-87-9	Fentin	5.79			0.2		
35554-44-0	Imazalil			42.4		12.4	
87820-88-0	Tralkoxydim	21.8				7.41	

[1]Data presented as active concentration (AC_{50}) in μM. Based on assay targets most relevant for effects on glucose control (i.e., excludes whole cell toxicity, genes involved in immune/inflammation)

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ToxRefDB Findings

Table 1. Selected results from ToxRefDB search for chemicals that caused increased body weight (or body weight gain), increased glucose, or pancreatic effects

ToxRefAppendix.xlsx

	A	B	C	D	E	F	G
1	Assay	Name (gene symbol	Official Full Name	AC_{50}	Fenthion		Return to
2	BSK BE3C uPAR up	PLAUR	plasminogen activator, urokinase receptor	1.48			
3	BSK hDFCGF PAI1 up	SERPINE3	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 3	1.48			
4	NVS ADME hCYP2J2	CYP2J2	cytochrome P450, family 2, subfamily J, polypeptide 2	1.65			
5	NVS ADME rCYP2C6	Cyp2c6	cytochrome P450, family 2, subfamily c, polypeptide 6	2.07			
6	NVS ADME hCYP2C19	CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	2.33			
7	NVS ADME hCYP1A2	CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	2.6			
8	BSK LPS MPC1 up	CCL2	chemokine (C-C motif) ligand 2	4.44			
9	NVS MP rPBR	Tspo	translocator protein (18kDa)	5.57			
10	NVS NR hAR	AR	androgen receptor	6.44			
11	NVS ADME hCYP2B6	Cyp2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	9.04			
12	ATG PXRE CIS	NR1I2	nuclear receptor subfamily 1, group I, member 2(Pregnane X Receptor)	10			
13	BSK BE3C hLADR up	HLA-DRA	major histocompatibility complex, class II, DR alpha	13.33			
14	BSK BE3C IL1a up	IL1A	interleukin 1, alpha	13.33			
15	NVS ADME hCYP3A5	CYP3A5	cytochrome P450, family 3, subfamily A, polypeptide 5	13.8			
16	ATG Ahr CIS	AHR	aryl hydrocarbon receptor	14			
17	ATG NRF2 ARE CIS	NFE2L2	nuclear factor (erythroid-derived 2)-like 2	18			
18	ATG PPRE CIS	PPARA	peroxisome proliferator-activated receptor alpha	18			
19	ATG PPRE CIS	PPARD	peroxisome proliferator-activated receptor delta	18			
20	ATG PPRE CIS	PPARG	peroxisome proliferator-activated receptor gamma	18			
21	NVS ADME rCYP2A1	Cyp2A1	cytochrome P450, family 2, subfamily a, polypeptide 1	18.3			
22	ATG ERE CIS	ESR1	estrogen receptor 1	26			
23	ATG VDRE CIS	VDR	vitamin D (1,25- dihydroxyvitamin D3) receptor	28			
24	ATG LXRa TRANS	NR1H3	nuclear receptor subfamily 1, group H, member 3(Liver X receptor alpha)	33			
25	ATG DR4 LXR CIS	NR1H2	nuclear receptor subfamily 1, group H, member 2(Liver X receptor beta)	34			
26	ATG DR4 LXR CIS	NR1H3	nuclear receptor subfamily 1, group H, member 3(Liver X receptor alpha)	34			
27	ATG ERa TRANS	ESR1	estrogen receptor 1	34			
28	ATG PPARg TRANS	PPARG	peroxisome proliferator-activated receptor gamma	36			
29	ATG LXRb TRANS	NR1H2	nuclear receptor subfamily 1, group H, member 2(Liver X receptor beta)	37			

Dimethomorph | Diniconazole | Diphenylamine | Disulfoton | Dithiopyr | Emamectin benzoate | Ethalfluralin | Ethofumesate | Famoxadone | Fentin | Fenthion

Sulfosulfuron (141776-32-1)	sulfonylurea	CHR, rat, feed	2.4	1296.5			244	Naylor 1997
Triasulfuron (82097-50-5)	sulfonylurea	SUB, rat, feed	10	1000		1000		Tai 1985
Tribenuron-methyl (101200-48-0)	sulfonylurea	CHR, rat, feed	1.25	62.5			62.5	Tobia 1987

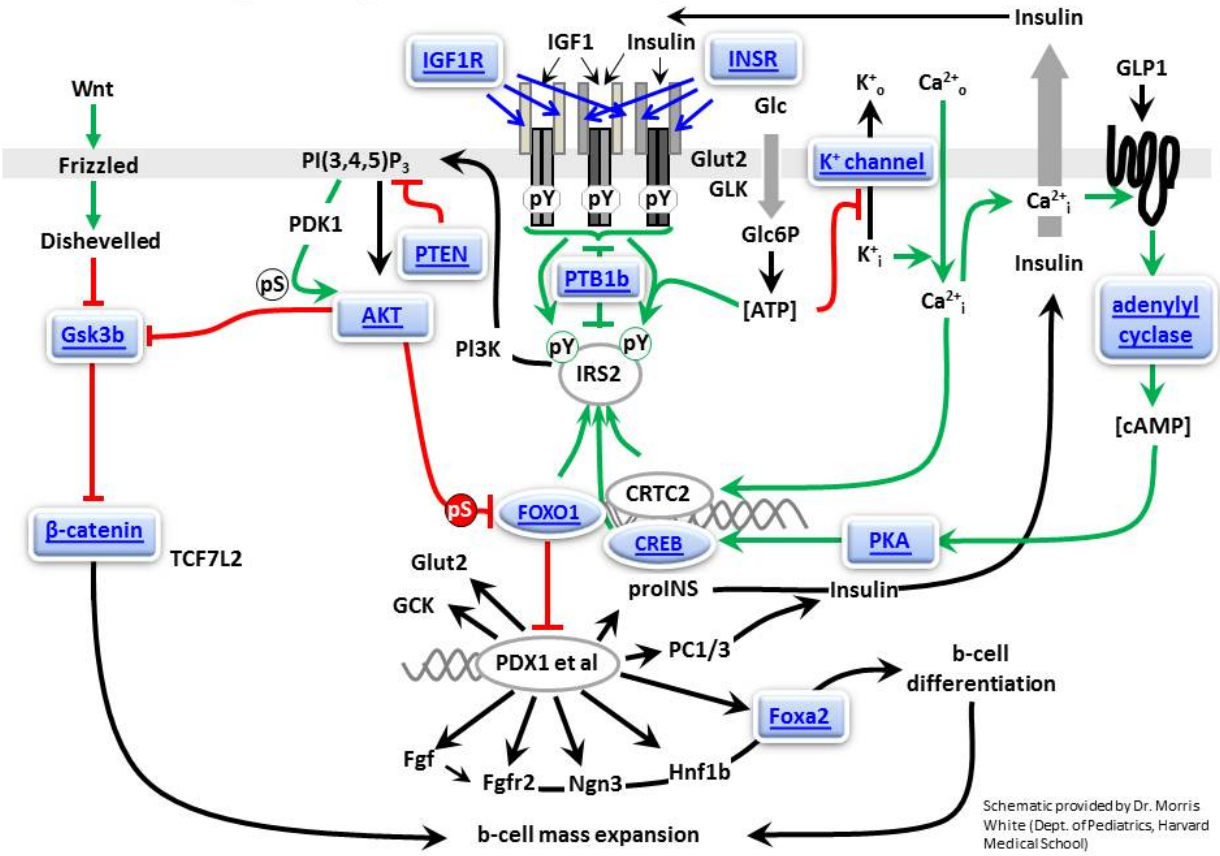
ToxCast™ and ToxRefDB

- ToxCast™ findings generally seemed to match strength of literature
  - Chemicals with strongest cases for biological plausibility would have been flagged, e.g., fentin and amitraz
  - Less consistent literature and less clear cut ToxCast "signal" for others, e.g., phthalates
- Provided biological support for organophosphate pesticides
- Suggested other chemicals should be tested based
  - "Hits" on signaling targets well-established to impact glucose homeostasis and adiposity, i.e., adrenergic receptors

Expert Input to Identify Most Relevant Tox21 Assay Targets

- Focus on biological processes
  - islet cell function, insulin sensitivity, adipocyte differentiation, feeding behavior
- Identify relevant assay targets already included in Tox21
- Identify assay targets to consider including

Insulin Signaling in Pancreatic β-Cells



Schematic provided by Dr. Morris White (Dept. of Pediatrics, Harvard Medical School)

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Sample Output From Signaling Hyperlinks to ToxCastDB

GSK3b

You are here: EPA Home > National Center for Computational Toxicology > ToxCastDB > Assay

ACToR | ToxRefDB | ToxCastDB | ExpoCastDB | DSSTox DB

Home | Basic Info | Data Collection List | Chemical List | Genes Associated with Assays | Help

Assay: Novascreen Human GSK3b

Assay Id:	914
Source	Novascreen
Source Name AID	NVS_ENZ_hGSK3b
Name	Novascreen Human GSK3b
Description	Human GSK3b Fluorescein-peptide
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
CATALOG NUMBER	200-0425
ASSAY CATEGORY	Enzyme Inhibition
ASSAY CATEGORY	In vitro (Biochemical)
ASSAY TARGET	GSK3b
ASSAY TARGET FAMILY	Kinase
ASSAY TARGET SOURCE	Recombinant
ASSAY GENE ID	2932
ASSAY GENE NAME	GSK3B
ASSAY TECHNOLOGY	Fluorescence -EMS
ASSAY REFERENCE COMPOUND	Staurosporine
ASSAY NOTE	KINASE
ASSAY SUBSTRATE NAME	CMGC group
ASSAY ATP CONCENTRATION (M)	NCCT_v2
ASSAY LIGAND NAME	1.5 E-06
ASSAY LIGAND CONCENTRATION (M)	1.20E-05
ASSAY BMAX	Fluorescein-peptide + ATP --> Fluorescein-phosphopeptide + ADP

Data

Name	CASRN	NVS_ENZ_hGSK3b (uM)
Mancozeb	8018-01-7	0.27
Maneb	12427-38-2	0.32
Metiram-zinc	9006-42-2	16.0

number of "actives" = 3

CREB

Assay: Attagene Factorial cis CRE

Assay Id:	16
Source	Attagene
Source Name AID	ATG_CRE_CIS
Name	Attagene Factorial cis CRE
Description	Factorial reporter gene assay
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
ASSAY URL	Link Out EXIT Disclaimer
ASSAY CATEGORY	In vitro (Cellular)
ASSAY TARGET	cAMP Response Element
ASSAY TARGET FAMILY	Transcription Factor
ASSAY TARGET SOURCE	Cell line
ASSAY TARGET SOURCE TYPE	HepG2
ASSAY GENE ID	10488
ASSAY GENE NAME	CREB3
ASSAY TECHNOLOGY	Reporter gene assay
ASSAY MODE	DNA sequencer
ASSAY REFERENCE COMPOUND	Forskolin cAMP
ASSAY NOTE	"Multiplexed reporter gene assay; cAMP, cGMP, NO receptor, GPCR pathways"

Data

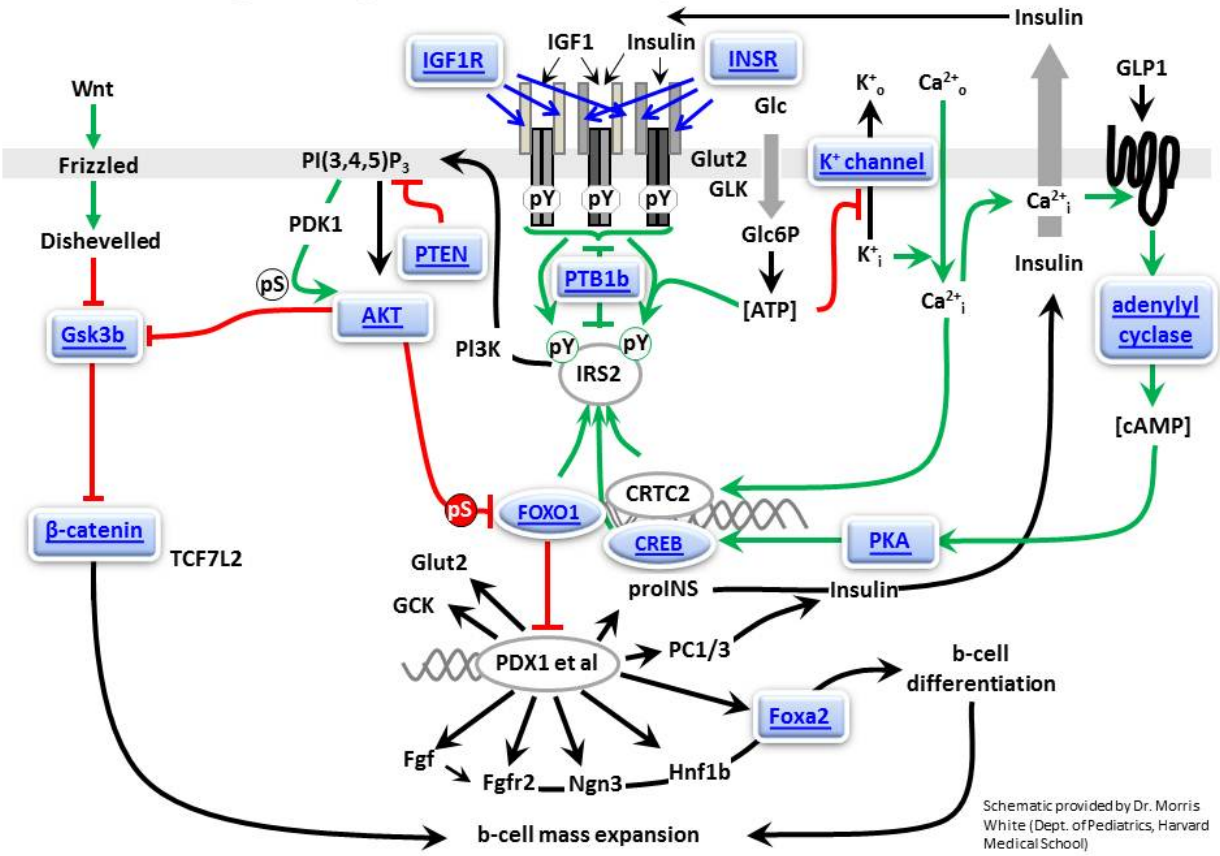
Name	CASRN	ATG_CRE_CIS (uM)
Alachlor	15972-60-8	3.4
Anilazine	101-05-3	59.0
Azinphos-methyl	86-50-0	27.0
Azoxystrobin	131860-33-8	46.0
Bendiocarb	22781-23-3	51.0
Bisphenol A	80-05-7	30.0
Bromoxynil	1689-84-5	40.0
Chlorpropham	101-21-3	31.0
Cyazofamid	120116-88-3	10.0
Cyprodinil	121552-61-2	23.0
Dazomet	533-74-4	49.0
Allethrin (d-cis,trans)	584-79-2	46.0
Dichloran	99-30-9	43.0

partial list:
number of total "actives" = 52

http://actor.epa.gov/actor/faces/ToxMiner/Home.jsp

EPA United States Environmental Protection Agency

Insulin Signaling in Pancreatic β-Cells



Schematic provided by Dr. Morris White (Dept. of Pediatrics, Harvard Medical School)

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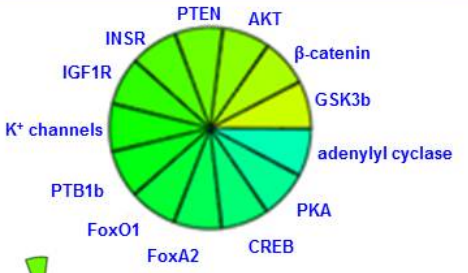
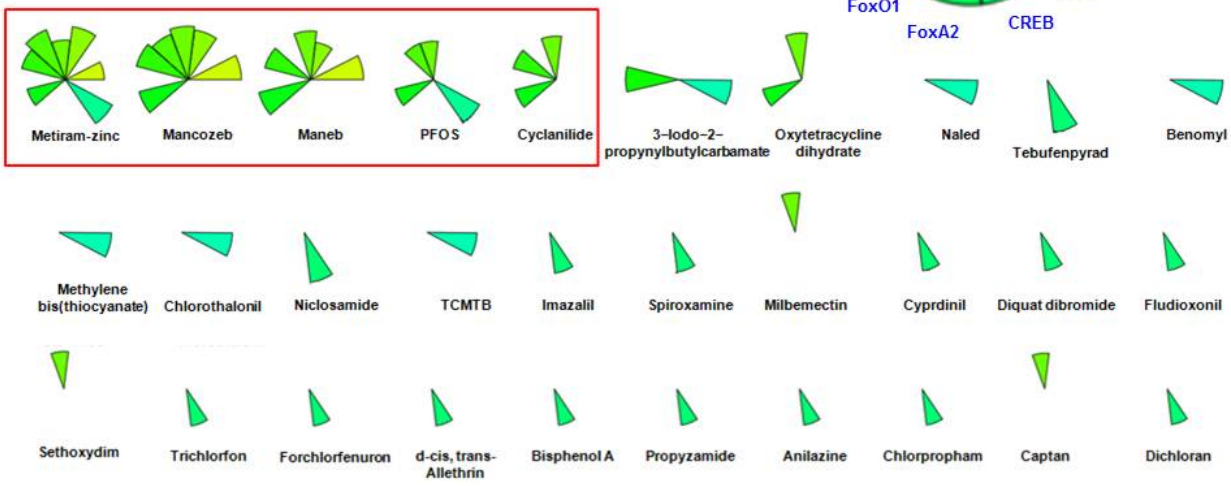
ToxPi™ for Insulin Signaling in Pancreatic β Cells- Top 30 from 309 Chemicals in ToxCast Phase I





http://epa.gov/ncct/ToxPi/

EPA United States Environmental Protection Agency

Targeted Testing Project

- Test HTS predictions in more physiological *in vitro* model systems*
  - Predicted "actives" and "inactives"

Islet cell function		Insulin sensitivity	Adipocyte differentiation	Feeding behavior	
A. Holloway*	**M. White***	**M. White**	**B. Blumberg*/ J. Schlezinger***	**D. Clegg (mammalian)**	**S. Srinivasan (C. elegans)***
Metiram-zinc	Mancozeb	Metiram-zinc	Tebupirimfos	Maneb	E. benzoate
Mancozeb	Metiram-zinc	Mancozeb	Prallethrin	Mancozeb	Fentin
Fentin	(Z,E)-Fenpyroximate	d-cis,trans-Allethrin	d-cis,trans-Allethrin	E. benzoate	Milbemectin
Milbemectin	Maneb	Spiroxamine	Fludioxonil	Metiram-zinc	Metiram-zinc
Maneb	Spiroxamine	Prallethrin	Cyazofamid	Bisphenol A	PFOS
HPTE	Imazalil	Niclosamide	Flusilazole	Milbemectin	Carbaryl
Chlorpyrifos oxon	Cyprodinil	PFOS	Fenthion	HPTE	HPTE
Cinmethylin	d-cis,trans-Allethrin	Tebufenpyrad	(Z,E)-Fenpyroximate	Cyazofamid	Amitraz
E. benzoate	Fipronil	Bromoxynil	Forchlorfenuron	Fentin	Chlorpyrifos oxon
Prochloraz	Thidiazuron	Cyclanilide	Fentin	Fluazinam	Chlorophene
Flusilazole	PFOS	Fentin	Tebufenpyrad	HPTE	Bendiocarb
Imazalil	Fludioxonil	Lactofen	Isazofos	Niclosamide	Naled
Chlorethoxyfos	Forchlorfenuron	Flusilazole	Dimethomorph	PFOS	Mancozeb
Bisphenol A	Trichlorfon	Quinoxyfen	Triadimefon	Chlorothalonil	Flusilazole
Naled	3-iodo-2propynly.	Diclofop-methyl	Diazinon	Fenthion	Thiocarb

Top Insulin-Sensitivity Gene Targets not in Tox21 (Morris White)

- Insulin receptor substrate-1 (IRS1)
- Insulin receptor substrate-2 (ISR2)
- Transcription factor 7-like 2 (TCF7L2)
- Phosphatidylinositol 3-kinase (PI3K)
- Phosphatase and tensin homolog (pTEN)
- Glucose transporter 2 (GLUT2)

Conclusions & NTP Follow-Up Activities

- General support for:
  - Plausibility of "obesogen" hypothesis
  - Linkage of diabetes to certain chemical exposures
    - Type 1 diabetes largely unexplored
  - Common mechanistic basis for certain chemical classes
- Follow-up
  - Submitting technical papers to Environmental Health Perspectives
  - Utilization of Tox21 approaches to identify substances of potential interest, i.e., targeted testing project
  - Assess human exposure to organotins
  - Assess ability to investigate environmental exposures in ongoing cohort studies
  - Meta Data Viewer as screening tool for human studies